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Neil Chernoff, Ellen Rogers, Brenda Carver, Robert Kavlock, and Earl Gray

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The Fetotoxic Potential of Municipal Drinking Water in the Mouse

NEIL CHERNOFF, ELLEN ROGERS, BRENDA CARVER, ROBERT KAVLOCK AND EARL GRAY
U. S. Environmental Protection Agency, Health Effects Research Laboratory, Research Triangle Park, North Carolina 27711

ABSTRACT Mice (CD-1 strain) were placed on diets containing either municipal drinking water (Durham, North Carolina) or water that had been distilled and passed through cartridges to reduce organics and remove inorganics. After a two-week acclimation period, animals were bred and pregnancy confirmed by the presence of a sperm plug. During the 8-month course of the study, approximately 500 pregnant mice were sacrificed on day 18 of gestation and their fetuses examined for visceral and skeletal anomalies. No significant water-related effects were found on any fetal parameter studied except for a 28.1% incidence of supernumerary ribs in the tap-water group as compared to 21.1% in the purified-water group. No differences were noted in the type or occurrence of anomalies between the two groups. A month to month variation was observed in a number of parameters. The degree of variation was similar for the treatment groups, suggesting that these changes might be random fluctuations.

The human population is exposed to numerous chemicals, identified and unidentified. Drinking water is one major source of exposure to diverse chemicals (Shackleford and Keith, '76; Junk and Stanley, '75). Research on the potential of such contaminants to alter the growth and development of mammals has been hampered by the sheer quantity of compounds, by the enormity of the problems involved in the identification and quantification of the contaminants, and by the possibility of synergistic and/or antagonistic actions which render studies done on single contaminants difficult to interpret.

McKinney et al. ('76) compared litters of CD-1 mice drinking Durham, North Carolina tap-water with mice drinking purified water and found significant increases in both fetal deaths and the number of litters with malformed fetuses in the tap-water group. The animals in this study were mated from November 1974 to March 1975 and the authors also noted that reproductive impairment had occurred in February 1973. The current studies were conducted in an effort to replicate this study during a similar period of the year.

MATERIALS AND METHODS

Random-bred 60-day old mice (CD-1 stock) [1] of both sexes were used. Upon arrival, mice were housed in plastic cages, with males being housed singly and females in groups of four. Cages were immediately assigned to either the tap-water (TW) or purified water (PW) groups. Animals were kept in rooms with controlled temperature (22-26°C) and lighting (12 hr of daily light). They were fed commercial lab chow and the designated water ad libitum.

The TW group received tap water from the municipality of Durham, North Carolina. The PW group received water that was generated by passing tap-water through an organic removal cartridge, demineralizer, and still.[2] Water was collected with polyvinyl and glass tubing and placed into glass containers. Water was administered in glass bottles which were rinsed with the specified water type before being filled and placed in the cages.

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[1] Charles River Breeding Laboratories, Wilmington, Massachusetts.

[2] Corning 3508-ORC organic removal cartridge; Corning 3508-B demineralizer; and a Corning "Meg-Pure" glass still.

After remaining on their assigned water groups for a minimum of 2 weeks, animals were bred within their specific groups. Four or fewer females were placed with a male in the afternoon and checked for vaginal plugs the following morning. Upon finding a plug (day 1 of pregnancy), the animal's ear was clipped for identification, it was weighed, and placed with a maximum of 4 other females successfully bred on the same day. The final percent pregnant was calculated as the number found pregnant at day 18 divided by the number of animals with vaginal plugs on day 1.

On day 18, animals were weighed and then killed by decapitation. The uterus and liver were removed and weighed. Maternal weight differences during gestation were calculated as the difference between the day 1 weight and the day 18 weight from which the gravid uterus weight was subtracted. The liver/body weight ratio was calculated using the corrected day 18 weight. Fetuses were removed and the uterus examined for resorption sites. All fetuses were examined for external defects. Live fetuses were weighed and randomly placed into either Bouin's fixative or 70% ethanol. Fetuses in Bouin's fixative were necropsied. Fetuses preserved in alcohol were eviscerated and subsequently cleared in 1% KOH and stained with alizarin red S to facilitate skeletal examination. During skeletal analysis extra ribs are defined as greater than or equal to 1/2 the length of the thirteenth rib, while rudimentary ribs are smaller, often being a barely discernible point of ossification (Kimmel and Wilson, '73). Fetal examinations were done by one individual throughout the experiment and performed on an experimenter-blind basis.

In those instances where vaginal plugs were not identified in mated females, dams were allowed to litter and randomly designated offspring were examined for skeletal anomalies as noted above.

The data were analyzed using a two-way analysis of variance procedure on the Statistical Analysis System (Barr et al., '76). A general linear models procedure was used to estimate the effects of the municipal versus purified drinking water on each independent variable. Since the experiment was conducted over a period of 8 months, with females being randomly assigned to water treatments, each "month" (referring to the month in which the vaginal plug was found) was considered a block in the statistical analyses. The data were analyzed by month when a possible ($p < 0.10$) water or month effect was observed. This was done to determine if the water effects were consistent or if the month effects were random or seasonal. Analysis of covariance procedure (Snedecor and Cochran, '71) was performed with two covariables (initial maternal weight and maternal weight gain) because the dams began pregnancy at significantly different weights (table 1) and the initial weight is highly correlated with maternal weight gain ($r = -0.41$; $p < 0.0001$). The analysis of covariance indicated that the initial maternal weight and the maternal weight gain were significantly related to many developmental parameters (the number of dead fetuses, the average fetal weight, and the degree of skeletal ossification). If differences in initial maternal and in average fetal weight were observed, then the differences in fetal weight could be due to the differences in the initial weight of the dams and not to the treatment regimen. Covariance analysis statistically adjusts the data as if each mother has an identical initial weight and maternal weight gain. Therefore, for each independent variable, the effects of water, month, and the

TABLE 1

Comparison of drinking and purified water regimens on CD-1 mouse development

	Purified water [a]	Drinking water [a]
Maternal observations		
No. inseminated	304	284
No. pregnant	257	236
% pregnant	85	83
Initial wt. (g)	33.7 ±0.2	34.1 ±0.2 [c]
Avg. wt. gain (g)	4.9 ±0.1	4.4 ±0.2 [d]
Avg. liver/body wt.	6.9 ±0.1	6.8 ±0.1
Fetal observations		
Avg. implants	12.1 ±0.2	12.4 ±0.2
Avg. live	10.4 ±0.2	10.4 ±0.2
Avg. dead	1.8 ±0.1	2.0 ±0.2
Avg. mortality (%)	14.7 ±1.1	15.4 ±1.2
Avg. weight (g)	0.97±0.01	0.95±0.01
Avg. sternals	5.5 ±0.1	5.4 ±0.1
Avg. caudals	3.3 ±0.1	3.2 ±0.1
Avg. supernumerary ribs (%)	21.1 ±1.7	28.1 ±1.9 [c]
Enlarged renal pelvis (%)	3.3 ±0.6	4.9 ±0.9
Cleft palate [b]	3/4	4/4
Micrognathia [b]	0/0	1/1
Encephalocele [b]	1/1	1/1

[a] Values given as Mean ± SEM.
[b] No. litters/no. fetuses.
[c] $p = < 0.05$.
[d] $p = < 0.01$.

TABLE 2

Comparisons of drinking and purified water regimens on CD-1 mouse development

	Month							
	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
PURIFIED WATER								
Maternal observations								
No. inseminated	51	55	21	60	18	37	43	19
No. pregnant	43	49	16	55	15	31	34	14
% pregnant	84	89	76	92	83	84	79	74
Initial wt. (g)	35.3 ±0.3	33.1 ±0.5	34.4 ±0.6	30.6 ±0.2 [c]	33.7 ±0.5 [a]	36.4 ±0.5	33.2 ±0.4	36.8 ±0.7
Avg. wt. gain (g)	4.6 ±0.3	4.6 ±0.3	4.2 ±0.5	6.7 ±0.2	4.8 ±0.4	3.0 ±0.4	6.3 ±0.3	2.4 ±0.8
Avg. liver/body wt.	7.1 ±0.1	6.9 ±0.1	7.2 ±0.2	7.3 ±0.1	6.1 ±0.2	6.6 ±0.2	6.5 ±0.1	6.7 ±0.1
Fetal observations								
Avg. implants	13.3 ±0.3	12.1 ±0.5	12.4 ±1.0	11.9 ±0.3	11.9 ±0.4	11.3 ±0.7	12.6 ±0.5	11.2 ±0.9
Avg. live	11.5 ±0.6	10.2 ±0.5	10.6 ±0.9	10.9 ±0.4	9.0 ±0.9	9.6 ±0.7	10.8 ±0.5	8.6 ±1.1
Avg. dead	1.8 ±0.2	1.9 ±0.2	1.8 ±0.4	1.0 ±0.2	2.9 ±0.9	1.7 ±0.5	1.8 ±0.3	2.6 ±1.0
Avg. mortality (%)	16.2 ±2.6	15.4 ±1.7	13.3 ±2.9	9.3 ±1.9	24.2 ±7.0	15.0 ±3.9	14.2 ±2.1	21.1 ±7.9
Avg. weight (g)	0.96±0.01	1.01±0.03	0.97±0.02	1.02±0.01 [b]	0.91±0.03	0.90±0.01	0.97±0.02	0.91±0.03
Avg. sternals	5.4 ±0.1	5.6 ±0.1	5.6 ±0.1	5.9 ±0.1	5.3 ±0.3	4.9 ±0.2	5.6 ±0.1	4.9 ±0.3
Avg. caudals	3.2 ±0.1	3.4 ±0.1	3.3 ±0.1	3.7 ±0.1	3.1 ±0.3	2.8 ±0.1	3.4 ±0.1	2.7 ±0.3
Avg. supernumerary ribs (%)	22.3 ±4.1 [a]	29.0 ±4.3	16.4 ±4.9	13.6 ±2.9 [b]	6.6 ±2.5 [c]	19.1 ±3.9	28.3 ±5.9	27.4 ±7.7
Enlarged renal pelvis (%)	0.0 ±0.0	6.3 ±1.9	1.9 ±1.3	4.2 ±1.2	1.7 ±1.7	1.7 ±1.0	5.7 ±2.1	1.1 ±1.1
DRINKING WATER								
Maternal observations								
No. inseminated	40	52	21	56	23	31	45	18
No. pregnant	31	41	21	46	20	26	38	13
% pregnant	78	79	100	82	87	84	88	72
Initial wt. (g)	34.8 ±0.4	33.0 ±0.6	34.4 ±0.5	32.5 ±0.4 [c]	35.3 ±0.5 [a]	36.3 ±0.5	33.0 ±0.4	36.3 ±0.7
Avg. wt. gain (g)	4.7 ±0.4	4.2 ±0.4	3.2 ±0.4	5.6 ±0.3	4.7 ±0.4	3.5 ±0.5	5.2 ±0.4	3.5 ±0.6
Avg. liver/body wt.	6.8 ±0.2	6.9 ±0.1	7.1 ±0.1	7.3 ±0.1	6.0 ±0.2	7.1 ±0.2	6.7 ±0.1	7.1 ±0.2
Fetal observations								
Avg. no. implants	13.0 ±0.7	12.6 ±0.6	11.4 ±0.7	13.1 ±0.5	11.5 ±0.7	12.2 ±0.7	12.6 ±0.4	12.2 ±0.5
Avg. live	10.9 ±0.7	11.0 ±0.6	9.4 ±0.8	10.5 ±0.5	10.1 ±0.7	9.6 ±0.7	10.7 ±0.3	9.6 ±1.2
Avg. dead	2.2 ±0.5	1.6 ±0.4	2.0 ±0.7	2.6 ±0.5	1.5 ±0.3	2.6 ±0.4	1.9 ±0.2	2.6 ±1.2
Avg. mortality (%)	15.1 ±3.2	11.6 ±2.6	17.4 ±5.3	18.3 ±3.2	13.0 ±3.3	20.8 ±3.3	14.1 ±1.7	20.8 ±9.4
Avg. weight (g)	0.94±0.01	0.97±0.01	0.96±0.03	0.96±0.01 [b]	0.92±0.02	0.93±0.01	0.94±0.02	0.93±0.02
Avg. sternals	5.3 ±0.1	5.5 ±0.1	5.3 ±0.2	5.4 ±0.2	5.2 ±0.2	5.5 ±0.2	5.3 ±0.1	5.5 ±0.1
Avg. caudals	3.4 ±0.1	3.5 ±0.1	3.2 ±0.1	3.3 ±0.1	3.2 ±0.2	3.2 ±0.1	3.2 ±0.1	3.2 ±0.2
Avg. supernumerary ribs (%)	38.5 ±6.6 [a]	23.3 ±3.8	28.1 ±6.1	27.6 ±4.4 [b]	24.6 ±6.8 [c]	24.5 ±5.1	30.1 ±4.8	24.5 ±8.2
Enlarged renal pelvis (%)	0.0 ±0.0	4.9 ±2.6	3.7 ±2.2	5.1 ±2.2	2.0 ±1.4	3.1 ±2.6	10.4 ±2.8	3.1 ±3.1

[a] $p < 0.05$.
[b] $p < 0.01$.
[c] $p < 0.001$.

interaction of water and months were estimated after correction for variation due to individual differences in initial weight and maternal weight gain.

RESULTS

The maternal and prenatal data are summarized on table 1. Although randomly assigned to treatment groups, the mean initial weights of the dams in the two groups were significantly different ($p < 0.04$). The animals in the TW group weighed 1.2% more than those in the PW groups. There also was a significant difference ($p < 0.04$) in the maternal weight gain during gestation, with PW animals having a greater increase (4.9 g) than TW animals (4.4 g). At the time of sacrifice, the maternal weights were not significantly different (PW = 38.6 g; TW = 38.5 g). No differences were noted in the incidence of pregnancy and liver/body weight ratios.

Analysis of the fetal parameters indicates no significant treatment-related effects in the number of implants, mortality, weight, or degree of ossification. There was a significant difference ($p < 0.005$) in the occurrence of supernumerary ribs (TW = 28.1%; PW = 21.1%). The distribution of ribs for both groups was approximately 40% rudimentary and 60% extra. Skeletal analysis of 60 day old offspring of mothers from both treatment groups yielded relatively similar percentages of supernumerary ribs. In the TW group, 35% of the pups examined (9/26) had supernumerary ribs as compared to 26% in the PW animals (9/34).

Visceral analysis showed no significant differences in either the type or occurrence of terata.

The month by month comparisons (table 2) were performed on those variables where a possible water effect was indicated ($p < 0.10$). The following significant differences between groups were found: % supernumerary ribs in August; initial maternal weight, avg. fetal weight, and % supernumerary ribs in November; and initial weight and % supernumerary ribs in December.

When the data were pooled for both treatments, significant variations also occurred on a monthly basis for most parameters studied (table 3). In those cases where "month" was a significant source of variance, the variation appeared to be random rather than seasonal.

TABLE 3

Maternal and fetal effects analyzed by two-way ANOVA with multiple covariance

	Month	Water	Month X water
Maternal liver/body weight	+++	ns	ns
No. implants	++	ns	ns
No. live	ns	ns	ns
No. dead	ns	ns	+
Average fetal weight	++	ns	ns
No. sternal ossif. centers	+	ns	ns
No. caudal ossif. centers	ns	ns	ns
% supernumerary ribs	ns	++	ns
% enlarged renal pelvis	++	ns	ns

ns not significant.
\+ significant, $p < 0.05$.
++ significant, $p < 0.01$.
+++ significant, $p < 0.001$.

DISCUSSION

The results reported here indicate that the developmental stages of mice are not sensitive to the municipal drinking water of Durham, North Carolina. There was a significantly reduced maternal weight gain during gestation in the TW group. In order to interpret the significance of this finding two factors must be considered. The first is that the TW animals were significantly heavier at the time of conception although no difference in the average time from arrival to successful mating was evident between TW and PW groups. The second factor is that the initial weight is inversely proportional to the weight gain during gestation. It is thus predictable that the TW animals would have gained less during gestation and, in fact, the average weights of the dams (excluding the uterine weight) at the time of sacrifice were equivalent (PW = 38.6 g; TW = 38.5 g).

The only significant water-related effect observed in the fetuses was an increased incidence of supernumerary ribs in the TW group. This effect was not consistent during the course of the study since analysis of the data by month indicates that the incidence of supernumerary ribs was higher in the PW animals in 2 of the 8 months studied. The biological significance of the anomaly is unknown.

When the data is analyzed on a monthly basis it becomes apparent that there is considerable variation in all the parameters studied regardless of treatment. For example, the average percent fetal mortality in the month of November was 9.3 in the PW group as com-

pared to 18.3 in the TW animals. In December, the situation was reversed with 24.2% mortality in the PW group and only 13.0% in the TW animals. While fluctuations in the TW group could reflect differences in drinking water quality, this would not be expected in the group receiving water of closely controlled quality. When coefficients of variation are compared we find that of the 13 variables measured, the coefficients of variation were greater in the PW group 6 times indicating that such fluctuations are random events rather than related to the water quality.

The experiments detailed in this report do not appear to substantiate the previous work of McKinney et al. ('76). We found no water-related differences in fetal mortality or the occurrence of fetal malformations, either in the overall study or for the months of November through March where differences were found by these workers. It should be remembered that the studies were done in different years and it is possible that the quality of the Durham drinking water has changed during the intervening years. The apparent discrepancy between studies may also be in part explainable by large month to month variation which was observed in both treatment groups in our experiment. Such variations which appear to be due to random fluctuation may result in interpretive errors in small population samples.

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